
Chapter 13

Nuclear-Mitochondrial Intergenomic Communication Disorders

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Additional information is available at the end of the chapter

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1. Introduction

Mitochondria are dynamic subcellular organelles present in virtually all eukaryotic cells with numerous functions. The most important of these functions is production of ATP; however they play an important role in various metabolic and developmental processes such as calcium homeostasis, apoptosis and programmed cell death, just to mention some. Mitochondria produce ATP by means of the mitochondrial respiratory chain (MRC) and oxidative phosphorylation (OXPHOS) system, a series of five enzyme complexes embedded in the inner mitochondrial membrane. Mitochondrial disorders most often refer to the dysfunction of OXPHOS system leading to deficiency in the ATP production. They are a group of genetically and phenotypically heterogeneous disorders with an incidence estimated to be between 1:5,000 and 1:10,000 live births [1].

MRC is the result of the interplay of two physically and functionally separated genomes, the nuclear DNA (nDNA) and mitochondrial DNA (mtDNA). Thirteen of the key structural polypeptides that constitute the multimeric subunits of the respiratory chain complexes are mtDNA encoded, in addition two ribosomal RNA (rRNA) and 22 transfer RNA (tRNA) that are required for initiating translation and protein synthesis [2]. Approximately 90 of the remaining proteins that make up the respiratory chain complexes are encoded by nDNA. Therefore, although human mtDNA encodes the basic machinery for protein synthesis, it depends entirely on the nucleus for the provision of enzymes for replication, repair, transcription, and translation. This dependency lies at the heart of several newly recognized human diseases that are characterized by secondary abnormalities of mtDNA.

The crosstalk between the two genomes is crucial for the cellular regulation of mtDNA integrity and copy number and correct mitochondrial protein production therefore mutations in genes involved in mitochondrial replication and maintenance can disrupt the

integrity of the mitochondrial genome, causing inter-genomic communication disorders. Multiple deletions, depletion of mtDNA or a combination of both phenomena (qualitative/quantitative lesions) in critical tissues, are the hallmarks of these disorders.

The focus of this chapter is to review the clinical and molecular etiologies of nuclear defects involved in mtDNA stability and in mitochondrial protein synthesis. The overview done here will hopefully provide insights towards best diagnostic strategies of mitochondrial cross-talk disorders, being useful for clinicians when facing similar cases. Additionally we will present a diagnostic algorithm for these diseases based on our knowledge.

2. Clinical manifestations of disorders affecting mtDNA integrity

Maintenance of mtDNA is controlled by an intricate homeostatic network, whose effectors are the various components of the mitochondrial replicosome and the many enzymes and carrier proteins that provide the mitochondrion with a balance supply of deoxyribonucleotides (Figure 1). As all of the factors are nDNA encoded, it is not surprising that mutations in genes involved in mitochondrial replication and maintenance can disrupt the integrity of the “tiny” mitochondrial genome [3] leading to multiple deletions or depletion [4]. The mitonuclear crosstalk has gained increased relevance in the past years and since then many genes have been identified as being involved in these diseases.

In the following section we will briefly review the clinical manifestations of both these group of disorders.

2.1. mtDNA multiple deletion syndromes

Mitochondrial diseases associated with the presence of multiple deletions of mtDNA are mostly autosomal dominant, occurring most often in adulthood. The size and terminals deletions are variable from one individual to another within the same family.

The main clinical manifestations associated with multiple deletions are:

- i. **PEO** (autosomal dominant or recessive **P**rogressive **E**xternal **O**phthalmoplegia). The most common clinical features include adult-onset of weakness of the external eye muscles, bilateral ptosis, proximal muscle weakness wasting and exercise intolerance. Additional symptoms are variable, and may include cataracts, hearing loss, sensory axonal neuropathy, ataxia, depression, hypogonadism, and Parkinsonism. Less common features include mitral valve prolapse, cardiomyopathy, and gastrointestinal dysmotility. Both autosomal dominant and autosomal recessive inheritance can occur; autosomal recessive inheritance is usually more severe [5,6]. The multiple deletions associated with PEO are exclusively found in muscle tissues of patients.
- ii. **SANDO** (Sensory Ataxic Neuropathy, Dysarthria and Ophthalmoparesis) is an autosomal recessive systemic disorder characterized mainly by adult onset of sensory ataxic neuropathy, dysarthria, and ophthalmoparesis. The phenotype varies widely, even within the same family, and can include myopathy, seizures, and hearing loss, but the common clinical feature appears to be sensory ataxia [7].

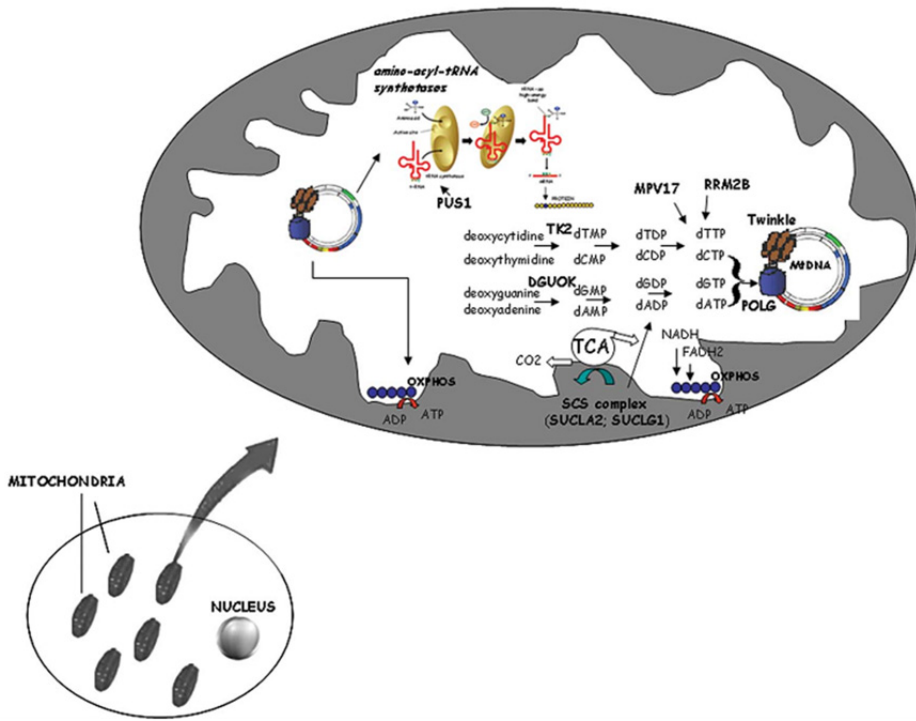


Figure 1. Schematic overview of the mitochondrion and the mitochondrial disease genes involved in intergenic communication disorders. Zooming in on the mitochondrion allows identification of genes (namely, *POLG* and *C10orf2*- *Twinkle*) thought to be involved in replication of mitochondrial DNA (mtDNA); those assumed to affect the metabolism of the mitochondrial deoxynucleotide (dNTP) pool (via progressive phosphorylations of deoxythymidine, deoxycytidine, deoxytadenine, and deoxiguanosine); and those belonging to the tricarboxylic acid cycle and affecting the respiratory chain complexes (OXPHOS). Moreover, the supposed role of genes involved in the complex machinery of mitochondrial protein synthesis (including the aminoacyl-tRNA synthetases) is illustrated. This figure was kindly provided by Prof. Filippo M. Santorelli.

- iii. **MNGIE** (Mitochondrial NeuroGastroIntestinal Encephalomyopathy), an autosomal recessive disorder clinically characterized by onset between the second and fifth decades of life, PEO, gastrointestinal dysmotility (often pseudo-obstruction), cachexia, diffuse leukoencephalopathy, peripheral neuropathy and early death. Mitochondrial DNA abnormalities can include depletion, multiple deletions, and point mutations [8].
- iv. **SCAE** (SpinoCerebellar Ataxia – Epilepsy syndrome) disorder similar to SANDO but with a higher frequency of migraine headaches and seizures [9].

2.2. mtDNA depletion syndromes

Quantitative alterations are characterized by depletion of mtDNA. Mitochondrial DNA depletion syndrome (MDS) comprises a heterogeneous group of autosomal recessive

disorders, all having the same molecular end result, low mtDNA amount in specific tissues. MDS are a group of rare and devastating diseases that manifest typically, although not exclusively, soon after birth, determining early death usually in infancy or early childhood. MDS differs from other respiratory chain disorders, as most often it may manifest solely in a specific organ (most commonly muscle or liver) [10]. However, it may occur that multiple organs, including heart, brain, and kidney are affected [11]. An extensive review on MDS was recently published [12].

Three major clinical categories can be recognized however, the clinical phenotypes are heterogeneous, overlapping and ever expanding [10,13]:

- i. **Hepatocerebral MDS** is most probably the most common variant of MDS; Onset of symptoms is between birth and 6 months; death usually occurs within one year of age. The most common symptoms and signs include persistent vomiting, failure to thrive, hypotonia and hypoglycemia associated with progressive neurological symptoms. Histological changes on liver biopsy include fatty degeneration, bile duct proliferation, fibrosis, and collapse of lobular architecture. Reduced COX histochemistry and combined deficiency of mtDNA encoded MRC complexes were found in the liver of a few patients.

A peculiar form of hepatocerebral MDS is Alpers-Huttenlocher syndrome, an early onset, fatal disease, characterized by hepatic failure, intractable seizures, evolving into *epilepsia partialis continua*, and global neurological deterioration. The liver dysfunction is usually progressive as well, evolving from microvesicular steatosis with bile duct proliferation into cirrhosis and chronic liver failure.

- ii. **Myopathic MDS** typically onset of symptoms usually occur in the first year of life with feeding difficulty, failure to thrive, hypotonia, muscle weakness and occasionally PEO. Death is usually due to pulmonary insufficiency and infections, but some patients survive into their teens [14,15]. Muscle biopsy may show proliferation of mitochondria, which can increase with age, and patchy or diffuse COX deficiency. Biochemical defects of all mtDNA-related respiratory chain complexes are always present in muscle mitochondria. Serum CK levels may be variably elevated [4].
- iii. **Encephalomyopathic MDS** is characterized by infantile onset of hypotonia with severe psychomotor retardation, high lactate in blood, progressive neurologic deterioration, a hyperkinetic-dystonic movement disorder, external ophthalmoplegia, deafness, generalized seizures and variable renal tubular dysfunction. Brain MRI was suggestive of Leigh syndrome [11].

3. Molecular etiologies of disorders affecting mtDNA integrity

In the next sections we will mention the genes identified so far, to be responsible with these disorders. Table 1 summarizes the mutations described and the associated phenotypes.

Gene	Phenotype	Mutation Type							
		M/N	Sp	Sd	Si	Sid	Gd	Gi	Gr
<i>POLG</i>	PEO	61		1	2				
	Alpers	37	5	2	6	2	3		
	MDS	6		1					
	Encephalopathy	3							
	Mitochondrial spinocerebellar ataxia and epilepsy	1							
	SANDO	1			1				
	POLG deficiency	29	1	2	2				
	OXPHOS deficiency	2							
	Ataxia	6	1						
	Epilepsy	2		1					
	Complex I deficiency	1							
	Others	18	1						
<i>POLG2</i>	PEO	1							
	Mitochondrial disease	7		1				1	
<i>SLC25A4</i>	PEO	5							
	Mitochondrial myopathy & hypertrophic cardiomyopathy	1	1	1	1				
<i>SLC25A3</i>	Muscular hypotonia & hypertrophic cardiomyopathy	1	1						
<i>C10orf2</i>	PEO	34				1		1	
	MDS	1							
	Spirocerebellar ataxia, infantile onset	2							
	Cholestatic liver disease	1							
	Encephalopathy	1							
	Ocular myopathy	1							
	Complex I deficiency	1							
	Dementia	1							
	Reduced expression	1							
<i>Tymp</i>	MNGIE	47	11	11	6	1			
	MDS	20	1	2	9	1	1		1
<i>TK2</i>	Epileptic encephalomyopathy				1				
	MDS with hearing loss				1				
	PEO	1							
<i>DGUOK</i>	MDS	28	6	9	4		1		
	MDS	12	2	1					
<i>RRM2B</i>	PEO	7		1	1				
	MNGIE	2							
	KSS	1							

Gene	Phenotype	Mutation Type							
		M/N	Sp	Sd	Si	Sid	Gd	Gi	Gr
<i>MPV17</i>	Altered p53 binding, association with MDS – hepatocerebral	7	1	1		1	2		
	Liver failure in infancy			2					
	Lethal hepatopathy & leukodystrophy		1						
<i>SUCLA2</i>	MDS – encephalomyopathic & methylmalonic ac.	4	1			1			1
<i>SUCLG1</i>	Succinyl-coenzyme A synthetase deficiency	4	1	1					
	Lactic acidosis with mitochondrial DNA depletion	2		1					
	Neonatal lactic acidosis with methylmalonic aciduria	2							
	Lactic acidosis, fatal infantile		1						
	Mitochondrial hepatoenkephalomyopathy	1							
<i>PUS1</i>	Mitochondrial myopathy and sideroblastic anaemia	2							
	Sideroblastic anaemia	1							
<i>TRMU</i>	Infantile liver failure, increased risk	6	1	1					
	Combined OXPHOS deficiency				2				
	Phenotype modifier	1							
	Respiratory chain deficiency, reversible infantile		1						
<i>LRPPRC</i>	Cytochrome c oxidase deficiency	1				1			
<i>TACO1</i>	Cytochrome c oxidase deficiency				1				
<i>TUFM</i>	Combined OXPHOS deficiency	1							
<i>TSFM</i>	Combined OXPHOS deficiency	2							
<i>GFM1</i>	Combined OXPHOS deficiency	8		2					
<i>MRPS16</i>	MRC disorder	1							
<i>RARS2</i>	Pontocerebellar hypoplasia	3	1	1					
<i>DARS2</i>	Leukoencephalopathy, brain & spine involvement, lactate elevation	15	11	1		2	1		
	Episodic ataxia, exercise-induced	1							
<i>YARS2</i>	MLASA syndrome	1							

Table 1. Mutations types described in genes involved in mtDNA integrity and mitochondrial translation and associated clinical phenotype (M/N- missense/nonsense; Sp- splicing; Sd- small deletions; Si- small insertions; Sid- small indels; Gd- gross deletions; Gi- gross insertions; Gr- gross rearrangements) - source HGMD Professional database www.hgmd.cf.ac.uk/.

3.1. Genes involved in mitochondrial replisome

3.1.1. *POLG*

Human mitochondria contain a single DNA polymerase, Polymerase gamma ($\text{POL}\gamma$), nuclear encoded and solely responsible for mtDNA replication and repair in mitochondria. $\text{POL}\gamma$ is composed of a catalytic subunit, $\text{POL}\gamma\text{A}$, which possesses both polymerase and proofreading exonuclease activities and an accessory subunit, $\text{POL}\gamma\text{B}$, which increases enzyme processivity [16]. The $\text{POL}\gamma$ holoenzyme functions in conjunction with the mitochondrial DNA helicase and the mitochondrial single-stranded DNA-binding protein to form the minimal replication apparatus [17]. It was generally accepted that mutations within the mtDNA were the major cause of mitochondrial diseases; however this view is changing as several of these have been linked to ineffective mtDNA replication by $\text{POL}\gamma$.

Mutations affecting the catalytic subunit $\text{POL}\gamma\text{A}$, encoded by the nuclear gene *POLG* are a major cause of mitochondrial disease, being highly heterogeneous – PEO, Parkinsonism, AHS, MNGIE, SANDO and SCAE- and usually is associated with multiple mtDNA deletions [18]. *POLG* mutations have been shown to be associated with all types of inheritance. The unique features of mitochondrial physiology are in part responsible for this variability but *POLG* structure and function add to the riddle of how one gene product can demonstrate autosomal recessive and autosomal dominant transmission. $\text{POL}\gamma\text{A}$ is a key player in mtDNA maintenance that is absolutely necessary for mtDNA replication from an early stage in embryogenesis [19]

In adPEO due to *POLG* mutations (most frequent), prominent features are severe dysphagia and dysphonia, and, occasionally, a movement disorder including Parkinsonism, cerebellar dysfunction, and chorea. Recessive mutations of *POLG* are responsible for sporadic and arPEO, as well as the syndromes referred above. Mutations in this gene can be also associated to the hepatocerebral form of MDS, namely AHS [18].

The *POLG* gene is located at chromosome 15, comprises 23 exons spanning 18.55 Kb. The gene was identified in 1996 [20] but only in 2001 the first pathogenic mutation was described. Since then more than 150 mutations have been reported and *POLG* gene is considered a hot-spot for mutations in mitochondrial diseases [21].

3.1.2. *POLG2*

MtDNA is replicated by DNA polymerase gamma, which is composed of a 140-kD catalytic subunit (encoded by *POLG*) and a 55-kD accessory subunit (*POLG2*). The accessory subunit increases enzyme processivity therefore it is not surprising that failure in this processivity leads to the accumulation of mtDNA deletions.

The *POLG2* gene is located at chromosome 17, comprises 8 exons spanning 19.28 Kb. In 2006 the first pathogenic mutation was described as being a cause of adPEO [22]. Since then, 10 mutations in *POLG2* have been reported.

3.1.3. *C10orf2* (*Twinkle*)

The mitochondrial helicase/primase encoded by *C10orf2* gene is also responsible for the adPEO [23]. Mutations in *C10orf2* may be of variable severity, being associated with clinical presentations ranging from late-onset “pure” PEO, to PEO complicated by proximal limb and facial muscle weakness, dysphagia and dysphonia, mild ataxia, and peripheral neuropathy. Recessive *C10orf2* mutations were also described in patients with hepatocerebral form of MDS [24].

The *C10orf2* gene is located at chromosome 10; it comprises 5 exons spanning 6.38 kb. The first pathogenic mutation was reported in 2001 [23] to be associated with PEO and since then 45 pathogenic mutations have been reported.

3.2. Genes involved in the synthesis and supply of nucleotide pools

3.2.1. *SLC25A4*

This gene, coding for the muscle-heart-specific mitochondrial adenine nucleotide translocator (ANT) is a member of the mitochondrial carrier subfamily of solute carrier protein genes [25]. ANT is the most abundant mitochondrial protein and in its functional state, it is a homodimer of 30-kD subunits embedded asymmetrically in the inner mitochondrial membrane. The dimer forms a gated pore through which ADP is moved from the matrix into the cytoplasm. There are three recognized isoforms of this protein.

Mutations in this gene have been shown to be responsible for the adPEO and have been also associated with a relatively mild, slow progressive myopathy, with little or no extramuscular symptoms.

The *SLC25A4* gene was identified in 2000 [25], it is located at chromosome 4, comprises 4 exons spanning 4.04 Kb. The first pathogenic mutations were described in 2000 and since then only seven mutations have been reported (most of them associated with PEO).

3.2.2. *SLC25A3*

The *SLC25A3* gene codes for a mitochondrial phosphate carrier. A defect in this mitochondrial phosphate carrier has been described in two children with hypertrophic cardiomyopathy, muscular hypotonia, severe growth retardation and death in the first year of life [26].

The gene is located at chromosome 12, comprises 7 exons spanning 8.37 Kb. The first pathogenic mutations were described in 2007 [26] and since then only one more mutation has been reported.

3.2.3. *Tymp* (*ECGF1*)

The *Tymp* gene, responsible for MNGIE (Mitochondrial NeuroGastroIntestinal Encephalomyopathy), encodes the enzyme thymidine phosphorylase (TP), which is

involved in pyrimidines catabolism. Defects of TP result in systemic accumulation of thymidine and deoxyuridine, which leads to deoxynucleotide pool imbalance and mtDNA instability, resulting in the presence of multiple deletions and partial depletion of muscle mtDNA [27].

The *Tymp* gene is located at chromosome 22 it comprises 10 exons spanning 4.3 kb. The first pathogenic mutations were described in 1999 [27] and since then 65 mutations have been described as being associated with MNGIE.

3.2.4. *TK2*

Thymidine kinase (TK2) is an intramitochondrial pyrimidine nucleoside kinase that phosphorylates deoxynucleotides (dNTPs), such as: deoxythymidine, deoxycytidine, and deoxyuridine, thereby participating in the salvage pathway of deoxynucleotide synthesis in the mitochondria [28]. Mitochondrial dNTPs pools arise either through active transport of cytosolic dNTP or through salvage pathways. Both pathways are essential for the replication of mtDNA, since the mitochondrion is unable to synthesize dNTPs *de novo*. Mutations in the *TK2* gene on chromosome 16q22 affect primarily muscle tissue, with little or no effect on the liver, brain, heart, or skin. The typical manifestation of *TK2* mutations is a severe, rapidly progressing myopathy of infantile or childhood onset. The disease course is rapidly progressive, leading to respiratory failure and death in months or years, but milder phenotypes with slower progression and longer survival have been reported [10]. Since the first mutation was described in 2001 [29], approximately 25 different pathogenic mutations in *TK2* have been published so far, either as recessive homozygous or compound heterozygous mutations, and phenotypes may be explained by variable degrees of residual activity of the mutant enzymes.

3.2.5. *DGUOK*

Deoxyguanosine kinase is a 2-deoxyribonucleoside enzyme that catalyzes the first step of the mitochondrial deoxypurine salvage pathway, the phosphorylation of purine deoxyribonucleosides into the corresponding nucleotides deoxyguanosine and deoxyadenosine necessary for the maintenance of mitochondrial dNTPs pools [11,30]. The typical phenotype of mutations in the *DGUOK* gene, on chromosome 2p13, is characterized by neonatal onset of progressive liver disease and feeding difficulties, usually with neurological dysfunction (hypotonia, nystagmus, and psychomotor retardation), by the age of 3 months. Peripheral neuropathy and renal tubulopathy have occasionally been reported [31]. Depletion of mtDNA has been documented only in the liver and results in combined respiratory chain deficiencies in the liver, whereas the amount of mtDNA is usually normal in muscle and fibroblasts. Histological analyses of the liver biopsy show variable findings, typically microvacuolar steatosis, cholestasis, fibrosis, and cirrhosis. In most cases, there is a rapidly progressive liver disease and neurological deterioration, with death occurring by the age of 12 months or shortly thereafter [32]. The first pathogenic mutations was reported in

2001 [33], since then more than 80 affected patients from approximately 50 families have been reported, and over 40 different *DGUOK* mutations have been identified [10]. The infantile hepatocerebral form of MDS is the almost invariable clinical presentation. Genotype-phenotype correlation studies show that patients who harbor null mutations usually have early onset liver failure and significant neurological disease, including hypotonia, nystagmus, and psychomotor retardation, and death before two years of age. Patients carrying missense mutations usually have isolated liver disease, a better prognosis, and longer survival.

3.2.6. *RRM2B*

The *RRM2B* gene on chromosome 8q23 encodes the small subunit of p53-inducible ribonucleotide reductase, a heterotetrameric enzyme responsible for *de novo* conversion of ribonucleoside diphosphates into the corresponding deoxyribonucleoside diphosphates that are crucial for DNA synthesis [34]. The enzyme is the main regulator of the nucleotide pools in the cytoplasm, and its small subunit is expressed in postmitotic cells, where it probably has a key function in maintaining the mitochondrial dNTPs pools for mtDNA synthesis. Mutations in *RRM2B* usually result in hypotonia, lactic acidosis, failure to thrive, and tubulopathy in the first months of life. The disease has a rapid progression and leads to death in a few months. The associated complex phenotype suggests that the consequences of a defective mitochondrial dNTPs pools can vary dramatically depending on the residual amount of the functional enzyme. Recently, it has been shown that inactivating mutations in *RRM2B* also cause severe neonatal or infantile forms of mtDNA depletion, with profound reduction of mtDNA copy numbers in skeletal muscle [34]. The first pathogenic mutation was reported in 2007 [34] and since then 26 mutations have been described.

3.2.7. *MPV17*

The *MPV17* gene is located on chromosome 2p23-p21 and encodes a mitochondrial inner membrane protein of unknown function recently recognized as responsible for mtDNA depletion. The clinical presentation is that of severe liver failure, hypoglycemia, growth retardation, neurological symptoms, and multiple brain lesions during the first year of life [35]. Marked mtDNA depletion in the liver is the molecular hallmark associated with multiple defects of respiratory chain complexes. Normal or mildly reduced levels of both mtDNA content and respiratory chain enzyme activities were also found in muscle [36]. Histological analyses of the liver have revealed swollen granular hepatocytes, microvesicular steatosis, and focal pericellular and periportal fibrosis. Since the first mutation was described in 2006 [37], about 15 different mutations have been reported in infantile-onset hepatocerebral syndrome and in Navajo neurohepatopathy, which is an autosomal recessive multisystem disorder found in the Navajo of the southwestern United States [30]. Three main subtypes are to be considered: infantile-onset (before 6 months) and

childhood-onset (before 5 years) forms with hypoglycemic episodes and severe progressive liver dysfunction requiring liver transplant, and a ‘classic’ form with moderate hepatopathy and progressive sensorimotor axonal neuropathy. The three forms are also associated with variable degrees of demyelination in both the central and the peripheral nervous system.

3.2.8. *SUCLA2 and SUCLG1*

Succinyl CoA synthase is a mitochondrial matrix enzyme that catalyzes the reversible synthesis of succinate and ATP or GTP from succinyl-CoA and ADP in the tricarboxylic acid cycle. This enzyme is made up of two subunits, a and b, encoded by *SUCLG1* on chromosome 2p11 and *SUCLA2* on 13q12, respectively. Mutations in *SUCLA2* and *SUCLG1* cause an encephalomyopathic form of infantile mtDNA depletion syndrome, but *SUCLG1* can also cause a very severe disorder with antenatal dysmorphisms, neonatal metabolic crisis, and early death, probably depending on the lower residual amount of the protein [38,39]. A useful diagnostic clue in Succinyl CoA synthase disorders of succinyl CoA synthase is a “mildly” elevated urinary methylmalonic acid, which is detected in all patients, and presence of tricarboxylic acid cycle intermediates (methylcitrate, lactate, carnitine esters, 3-hydroxyisovaleric acid) in most cases. Some patients die as infants (sudden infant death syndrome), but some of them have a longer survival. The clinical features of patients with mutations in these genes include early childhood hypotonia, developmental delay, and almost invariably, progressive dystonia and sensorineural deafness. *SUCLA2* and *SUCLG1* mutations seem to disrupt an association between succinyl CoA synthase and mitochondrial nucleoside diphosphate kinase, resulting in an unbalanced mitochondrial dNTP pool and eventually, mtDNA depletion in muscle. The first pathogenic mutations were reported in 2005 [40] and 2007 [41] in *SUCLA2* and *SUCLG1*, respectively and since then few mutations have been described.

3.3. Genes involved in mitochondrial translation

Mendelian diseases characterized by defective mitochondrial protein synthesis and combined respiratory chain defects have also been described in infants and are associated with mutations in nuclear genes that encode components of the translational machinery, such as those encoding elongation factors, aminoacyl-tRNA synthetases, or even mtDNA encoded tRNA [12]. Mitochondria contain a separate protein-synthesis machinery to produce the polypeptides encoded in mtDNA, and many mtDNA disease mutations affect this machinery. This group of disorders is highly heterogeneous and usually shares a combined disorder of respiratory chain complexes.

3.3.1. *Genes involved in mitochondrial translation factors*

3.1.1.1. *PUS1*

The Pseudouridine synthase 1 (*PUS1*) gene on chromosome 12q24 encodes an enzyme that converts uridine into pseudouridine at several cytoplasmic and mitochondrial tRNA

positions and thereby improves translation efficiency in the cytosol as well as the mitochondrion. Thus, *PUS1* is not part of the translation machinery, but it is required for protein synthesis because of its function in posttranscriptional modification of tRNA. Mutations in *PUS1* are responsible for the rare myopathy, lactic acidosis, sideroblastic anemia syndrome and sometimes include mental retardation. The first pathogenic mutation was reported in 2004 [42] and since then few mutations have been described.

3.1.1.2. *TRMU*

The *TRMU* gene on chromosome 22q13 encodes an evolutionarily conserved protein involved in mitochondrial tRNA modification and is important for mitochondrial translation. Defects in tRNA 5-methylaminomethyl-2-thiouridylate methyltransferase (*TRMU*), a mitochondria specific enzyme that is required for the 2-thiolation on the wobble position of the tRNA anticodon, result in reduced steady-state levels of 3 tRNA (tRNA^{Lys}, tRNA^{Gln}, and tRNA^{Glu}) and consequently, impaired mitochondrial protein synthesis [43,44]. Recently, mutations in *TRMU* were detected in patients with acute liver failure in infancy [44].

3.1.1.3. *LRPPRC*

The *LRPPRC* gene is located on chromosome 2p21. Leucine-rich PPR-motif containing protein has been suggested to function together with heterogeneous nuclear ribonucleoprotein K and RNA polymerase in coupling the mitochondrial transcription and translation machineries [45]. Mutations in *LRPPRC* lead to the French-Canadian subtype of Leigh syndrome, associated with a profound deficiency of complex IV of the OXPHOS system [46]. Patients exhibit neonatal or infantile onset hypotonia and psychomotor delay, and bilateral hyperlucencies of basal ganglia, like other more common forms of Leigh syndrome. The first pathogenic mutation was reported in 2003 [46] and since then one more mutation has been described.

3.1.1.4. *TACO1*

TACO1 represents the first specific mammalian mitochondrial translational activator, opening the possibility to a new class of proteins controlling efficiency of mitochondrial translation. Mutations in *TACO1*, located on chromosome 17q.6, are responsible for a relatively late-onset Leigh syndrome (onset range 4-13 years) characterized by short stature, mental retardation with autistic-like features, and a slowly progressive array of motor symptoms related mainly to basal ganglia involvement [47,48]. Only one mutation was described to date [47].

3.1.1.5. *TUFM*, *TFSM* and *GFM1*

Another important player during mitochondrial protein biosynthesis is the group of elongation factors. The mitochondrial EF-Tu forms a ternary complex with tRNA and GTP and promotes the binding of tRNA to the ribosome. A few patients have been described as

having mutations in genes encoding components of the mitochondrial translation elongation machinery, including elongation factor EF-Tu (TUFM), EF-Ts (TSFM) and EFG1 (GFM1). These patients have severe disease, presenting neonatal lactic acidosis and neurological impairment resembling Leigh syndrome, leading to early fatality. The first pathogenic mutations in these genes were reported recently [49,50,51] and since then few mutations have been described.

3.1.1.6. *MRPS16 and MRPS22*

Of all 81 human mitochondrial ribosomal proteins (MRPs), mutations have been found in only two, MRPS16 and MRPS22 [52,53]. Both defects resulted in a marked decrease in the 12S rRNA transcript level, probably caused by impaired assembly of the mitoribosomal small subunit, generating unincorporated and unstable 12S rRNA. Indeed, lack of MRPs results in the failure to assemble parts of small subunits of the mitoribosome, and subsequent degradation of its components [54]. Clinical manifestations include agenesis of the *corpus callosum*, dysmorphism, hypertrophic cardiomyopathy, and fatal neonatal lactic acidosis. The first pathogenic mutations were reported in 2004 [52] and in 2007 [53], and since then few mutations have been described.

3.3.2. Genes involved in mitochondrial aminoacyl tRNA synthetases

3.3.2.1. *RARS2, DARS2, and YARS2*

To guarantee fidelity in translation, it is important to attach the right amino acid to the tRNA and to ensure that the tRNA recognizes, through its anticodon, the correct codon in the ribosomal A-site. Incorporation of an incorrect amino acid into the nascent polypeptide could cause misfolding and production of defective or dominant interfering proteins. Amino acids are attached to tRNA by amino-acyl-tRNA synthetases, each of which is specific for a single amino acid. However, as there can be several codons and several different tRNA for a single amino acid, an amino-acyl-tRNA synthetase can “charge” several different tRNA. If this function is defective, certain codons will become ambiguous, resulting in the synthesis of misfolded proteins, which could aggregate to form inclusions and induce further protein misfolding. Mutations in the *RARS2* and *DARS2* were recently described [55,56,57] and are associated with severe encephalopathy with pontocerebellar hypoplasia and leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation, respectively, with most patients showing onset between 2-15 years of age [56]. Very recently, mutations in the gene encoding the mitochondrial *YARS2* have been associated with a clinical condition characterized by myopathy, lactic acidosis, and sideroblastic anemia [54].

4. Diagnostic approaches for intergenomic communication disorders

Suspicion of intergenomic communication disorders arising from clinical presentation may range from well defined syndromes to unspecific multisystemic phenotype, where neurological involvement is usually present.

Establishing a specific diagnosis in a patient with suspected mendelian disease is a challenging task that requires the integration of clinical assessments, family history, biochemical testing and histopathological examination. It is important to obtain the appropriate biochemical and/or clinical information before starting any molecular investigations so that molecular diagnosis can be successfully.

Biochemical determination of mitochondrial respiratory chain complexes is important for delineating the molecular approach in particular in patients without a specific neurological syndrome. As mtDNA encodes for subunits of respiratory chain complexes I, III, IV and the ATP-synthase, mtDNA depletion causes a combined respiratory chain deficiency of all complexes, except complex II. Biochemical analysis of the muscle respiratory chain enzyme activities may, however, be normal, if skeletal muscle is not among the affected tissues, e.g., in MDS of the brain or liver. Southern analysis or quantitative real-time polymerase chain reaction are two methods that simultaneously detects mtDNA deletion(s) and quantify total mtDNA content. In both approaches, mtDNA amount is compared to a specific nuclear reference gene. A prerequisite for correct interpretation of mtDNA amount is to consider the dynamic nature of mtDNA amount in different ages and tissues, and therefore to establish carefully age-matched control materials [58]. A reduction in mtDNA copy number to 60-65% of age-matched controls has been established for an empirical cut-off level for MDS diagnosis, but especially in children, the reduction may be severe (80-90%). Biochemical data, such as lactate, pyruvate, alanine, organic acid profiles as well as neuroimaging findings are also important clues for the diagnosis of these disorders. Some diagnostic clues exist for specific gene defects: serum creatine kinase (CK) is elevated in *TK2* defects, serum thymidine in *TYMP* defects and urine methylmalonic acid and methylcitrate in *SUCLA2* and *SUCLG1* defects [10].

The *POLG* gene seems to be the most frequently mutated nuclear gene in cases of mitochondrial disease therefore in cases of normal mtDNA testing and clinical signs such as nonspecific hypotonia, developmental delay, epilepsy and progressive liver disease *POLG* gene investigation should be considered. Valproate-induced liver toxicity in *POLG* and *C10orf2*-MDS emphasizes the importance of diagnosing these patients, who usually suffer from severe treatment-resistant epilepsy [59]. We suggest *POLG* analysis before valproate treatment for such children and adolescents, whose first epileptic attack develops to a status epilepticus of unknown cause.

Based on our practice, we present a testing algorithm for establishing an accurate diagnosis for these diseases (Figure 2).

5. Therapeutic considerations

The management of mitochondrial disease is largely supportive as no curative therapy is available. Palliative/supportive treatment with vitamins, cofactors and respiratory substrates have been used, but with poor efficacy. In the last years several approaches have been tried and the enhancement of mitochondrial biogenesis has emerged as an exciting therapeutic possibility. The enhancement of mitochondrial biogenesis might restore mitochondrial function in a variety of other contexts.

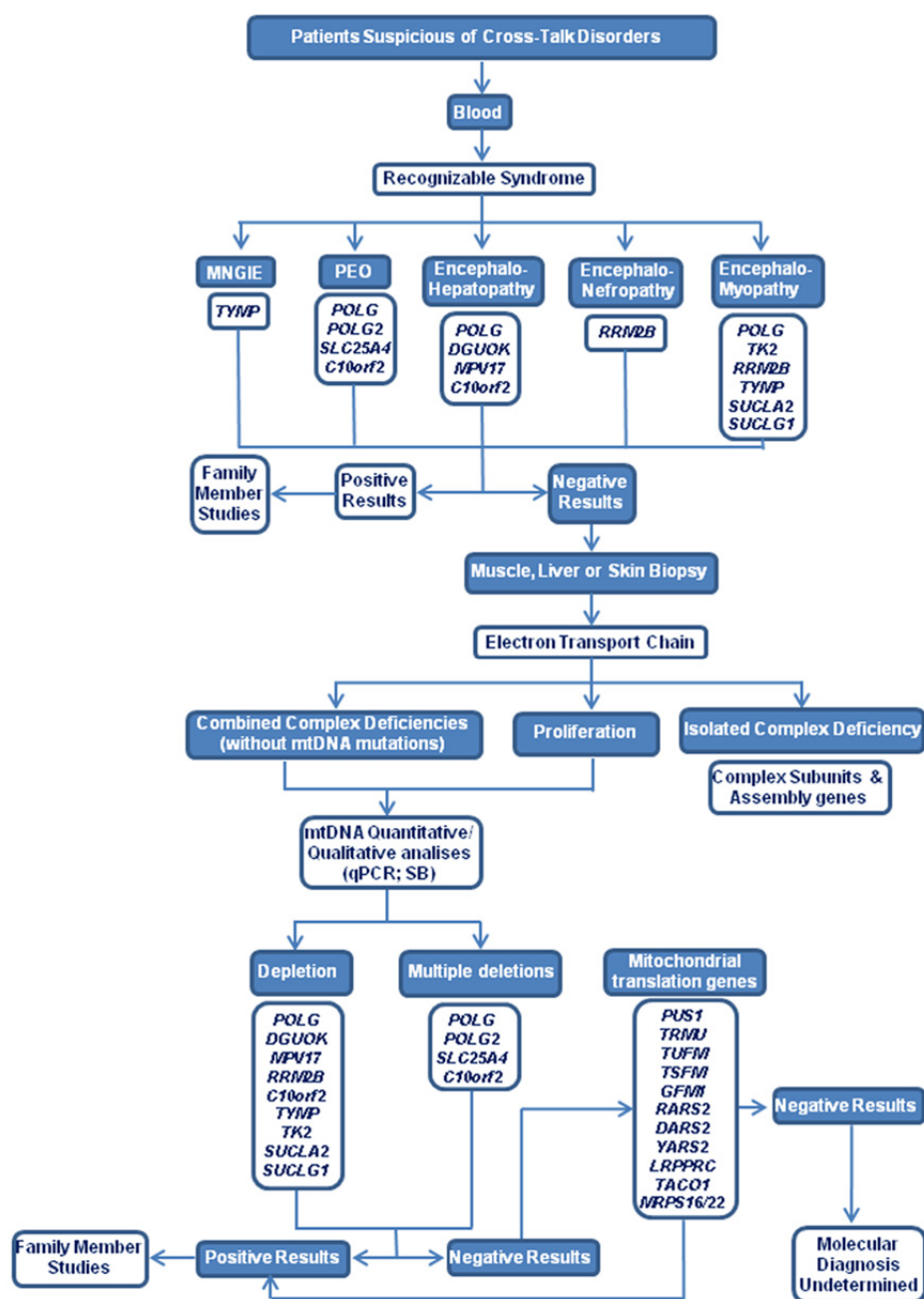


Figure 2. Diagnostic algorithm for intergenic communication disorders, based on clinical and biochemical information.

What has been noticed is that for every case there is a different strategy. For example liver transplantation may be beneficial to patients with hepatopathy caused by *DGUOK* mutations if no neurological symptoms have developed. However, significant hypotonia, psychomotor retardation or nystagmus should be contraindications for the liver transplantation [60]. In patients with *MPV17*, liver transplantation has increased quality and years to life for some patients [61,62], but the patients have developed neurological symptoms. Some children with *POLG* mutations have received a liver transplant after valproate-induced liver failure, and although it has rescued their liver function, neurological outcome has been unfavorable [63,64].

In patients with *MPV17* mutations, a controlled diet avoiding hypoglycemias were suggested to slow down the progression of liver impairment and be useful in supportive care [65]. Some improvement of liver functions in a patient with *MPV17* mutations was gained by treating them with succinate or coenzyme-Q10 together with a lipid-rich diet [66]. Further studies with larger patient materials and longer follow-up time are needed to confirm, if these dietary interventions were beneficial, and could be recommended. In MNGIE, correlation between plasma thymidine levels and the severity of the phenotype has been observed [67]. Therefore, attempts to reduce the circulating nucleotide levels could result in disease improvement. Enzyme replacement therapy has been applied for MNGIE: infusion of platelets from healthy donors to patients with MNGIE reduced their circulating thymidine and deoxyuracile levels, and partially restored TP activity. The limitation of this therapy was the short half-life of platelets [68]. Allogenic stem cell transfusions have been given to two patients with MNGIE [69]. Although more experience is needed to illustrate the clinical benefit of that treatment, it opens up a possibility of treatment for disorders of the nucleoside metabolism. In MNGIE, also continuous ambulatory peritoneal dialysis has been used to reduce the thymidine levels, and this resulted in improvement of the symptoms during 3-year follow-up time [70]. Good animal models will enable testing these hypotheses *in vivo*.

6. Conclusive remarks

The diagnostic process in nuclear disorders of oxidative metabolism is not too different from that employed for other diseases and includes patient and family history, physical and neurologic examination, routine and special laboratory tests, muscle biopsy for morphology and biochemistry, and molecular genetics screening [71]. A mitochondrial disease manifesting at or soon after birth is more likely to be associated with nDNA than with mtDNA mutations, but until very recently, our profound ignorance regarding the mechanisms underlying mitochondrial gene transcription and translation and the complex interaction between the “2 genomes” has limited our diagnostic power. Mitochondrial DNA deletion and depletion syndromes, and disturbances in the mitochondrial translation machinery have become an increasingly important cause of a wide spectrum of infantile and childhood-onset multisystem disorders. Depletion syndromes could result from any imbalance of the mitochondrial dNTPs pools available for mtDNA replication, as well as abnormalities in either the mitochondrial helicase or DNA polymerase. Consistent with the

different phenotypes, mtDNA depletion may affect specific tissue (most commonly, brain and muscle or liver) or multiple organs, including the heart and the kidney. Predictably, affected tissues show paucity of mtDNA-encoded translation products and multiple respiratory chain defects. More than 75% of these patients had onset during the first year of life, and the disease was rapidly fatal in most cases [3,72,]. Moreover, though the components of the complicated mitochondrial protein-synthesis machinery are exclusively nuclear encoded, the majority of mutation affects correct translation of mtDNA-encoded subunits of the OXPHOS system and accounts for a still undetermined number of genetic defects. Indeed, there is still limited information on the many mitoribosomal proteins; the several tRNA maturation enzymes; the aminoacyl-tRNA synthetases; the translation initiation, elongation, and termination factors; and the predictably larger number of unidentified factors needed for ribosome assembly [43,73].

The increasing number of nuclear governed mitochondrial diseases and its associated genes continues to increase the diversity of the genetic and clinical phenotypic heterogeneity of this group of disorders. Identifying the causative genes is not only important for adequate genetic counseling and prenatal diagnosis but also to have a better understanding of the disease pathophysiology leading to better therapy options. The increasing number of genes involved is a driving force for the development of high throughput strategies. The recent advances on sequencing technology will facilitate the molecular investigations of genes associated with mtDNA disorders in general. Reports concerning the use of next generation sequencing for the diagnosis of mitochondrial disorders are emerging [74,75,76]. In a recent report the use of target NGS for mitochondrial disorders proved its efficiency in clinical diagnosis as for 55% of the studied patients a clear molecular etiology was found. As more studies are reported the importance of applying this technology will be highlighted.

The problems faced by patients with mitochondrial respiratory chain disease are particularly severe. Diagnosis is difficult, treatment is largely ineffective, genetic counseling and prenatal diagnoses are uncertain or unavailable and the prognosis is unpredictable. Because diagnosis is imperfect and laborious, many patients undergo a whole battery of unnecessary investigations during the diagnostic process. Accurate focused diagnosis will save time, money and distress. Only by understanding the molecular genetic basis of these disorders, whether nuclear or mitochondrial, will any progress be made. Furthermore this will help patients, but will also lead to fundamental advances in our understanding of mitochondrial biology. Identification of new disease-causing gene(s) will hopefully provide insights towards novel therapeutic strategies.

Chapter highlights

- The chapter focus on diseases of intergenomic communication disorders mainly the ones involved in mtDNA integrity and mitochondrial protein synthesis
- Disorders affecting mtDNA stability lead to multiple deletions or depletion of mtDNA
- This group of disorders can affect a variety of organ with variable ages of onset
- *POLG* is frequently mutated being a hotspot for mitochondrial disease

- Diagnosis is difficult and laborious due to the increasing number of genes involved
- Therapy is mainly palliative however novel strategies are emerging
- Due to the increasing number of genes involved novel diagnostic strategies are emerging to optimize the diagnosis offered to these families

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